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Current WHO Guidelines and the Critical Role of Immunohistochemical Markers in the Subclassification of Non-Small Cell Lung Carcinoma (NSCLC). Moving from Targeted Therapy to Immunotherapy

Lais Osmani, MD, **Frederic Askin, MD**, **Edward Gabrielson, MD**, and **Qing Kay Li, MD, PhD**

Department of Pathology, Johns Hopkins Medical Institutions, Baltimore, MD 21224

Abstract

Recent large scale genomic studies from the Clinical Lung Cancer Genome Project have identified different driver gene mutations in the subtypes of non-small cell lung carcinoma (NSCLC). These findings not only lead to remarkable progress in targeted therapies for lung cancer patients, but also provide fundamental knowledge for the subclassification of NSCLC. More recently, the advancement and clinical application of immunotherapy have reinforced the need for the accurate subclassification of NSCLC. In 2015, the World Health Organization (WHO) and the International Association for the Study of Lung Cancer (IASLC) updated their guidelines for the subclassification of lung cancers. These guidelines emphasize: (1) the subclassification of NSCLC, (2) the critical role of molecular characterization of tumors for targeted therapy, (3) the unique terminology for subclassifying NSCLC using small biopsy specimens, and (4) the utility of IHC biomarkers in the accurate diagnosis and subclassification of lung cancer. The guidelines have significant prognostic impact on oncologic practice and patient care. In this review, we summarize the current WHO guidelines for the classification of lung cancer, discuss advancements of targeted therapy and immunotherapy, and address the utility and limitation of immunomarkers in the subclassification of NSCLC, as well as the prospective future of the field.

Keywords

non-small cell lung carcinoma (NSCLC); *EGFR* mutations; fine needle aspiration (FNA) biopsy; targeted therapy; PD-L1 immunotherapy

Background

Lung cancer is a heterogeneous group of tumors, consisting of more than 50 histomorphological subtypes [1–3]. Over the past few decades, non-small cell lung

Address Correspondence to: Qing Kay Li, MD PhD, Associate Professor, Department of Pathology, Johns Hopkins Bayview Medical Center, 4940 Eastern Ave., Building AA, Room 154B, Baltimore, MD 21224, Phone: (410) 550-0671, qli23@jhmi.edu.

carcinoma (NSCLC) and small cell lung carcinoma (SCLC) were the most frequently used diagnostic terms for lung cancer, mainly due to limited treatment options, which usually did not require further morphological subclassification. NSCLC comprises approximately 80–85% of all lung cancers [1, 2] with adenocarcinoma (ADC, approximately 40–50% of cases) and squamous cell carcinoma (SqCC, approximately 20–30% of cases) comprising the predominant histological subtypes of NSCLC [1–3]. Clinically, only a small portion of NSCLC patients are diagnosed at an early stage (stage I or II), when the tumor can be treated by surgical resection [2]. Over 60% of lung cancer patients present with locally advanced or metastatic disease (stage III or IV) at the time of diagnosis, at which point surgical resection may not be an option. Until recently, conventional chemotherapy and radiation therapy were the main stays of treatment for lung cancer patients.

Within the last decade, the discovery of *EGFR* (epidermal growth factor receptor) gene alterations and *EML4-ALK* (echinoderm microtubule-associated protein-like 4 and anaplastic lymphoma kinase) gene rearrangement in lung ADCs has led to the development of targeted therapy using TKIs (tyrosine kinase inhibitors) and crizotinib, respectively [4–7]. These targeted therapies have become the standard of care, and have led to improved clinical outcomes in a subset of lung cancer patients, whose tumors harbor *EGFR* or *EML4-ALK* alterations. The development of targeted therapies has advanced the therapeutic strategy from conventional chemo- and radiation-based therapy to genetic alteration-guided targeted therapy [8, 9]. Recently, large scale genomic studies from the Clinical Lung Cancer Genome Project have identified different driver gene alterations in certain subtypes of NSCLC. In ADC, *EGFR* activating mutations and *EML4-ALK* rearrangements are detected in approximately 25% of tumors. In addition, loss-of-function mutations in *LKB1/STK11*, *NF1*, *CDKN2A*, *SMARCA4* and *KEAP1*, mutations in *KRAS*, amplification of *MET*, and rearrangements of *ROS1* and *RET* have also been identified [10]. In contrast, SqCCs rarely harbor *EGFR* mutations or *EML4-ALK* rearrangements. Instead, SqCCs have alterations in *RTKs* , *DDR2* and *FGGRs,* as well as inactivating mutations in *CDKN2A, PTEN, KEAP1, MLL2, HLA-A, NFE2L2, NOTCH1* and *RB1* [11]. Based on genomic studies and analyses of molecular pathways in different subtypes of lung cancer, the development of targeted therapies and clinical trials have increased rapidly (Table 1).

Although targeted therapy has improved clinical outcomes in certain subsets of lung cancer patients, the 5-year survival rate is still less than 20% [1–3]. More recently, immunotherapy has been developed and increasingly used in lung cancer patients. For example, PD-L1 (programmed cell death ligand 1) is a key immunoregulatory molecule which, upon interacting with its receptor, PD-1, inhibits the CD8 cytotoxic immune response and the resultant antitumor immune response [12–14]. In a variety of tumors, including lung cancer, PD-L1 is over expressed on tumor cells and plays an important role in the modulation of the immune response to both the tumor and host cells. Currently, there are several clinical trials involving FDA-approved immune checkpoint inhibitors, which attack PD-L1 expressing tumor cells by blocking the PD-L1/PD-1 signaling pathway. The use of these agents not only requires accurate subclassification of tumor types, but also requires specific testing to assess the level of PD-L1 expression on tumor cells by IHC (immunohistochemical) stains [15, 16]. Different anti-PD-L1/PD-1 agents require different detection methods and assay conditions (Table 2). For example, pembrolizumab requires the detection of PD-L1 expression on

tumor cells by using the PD-L1 IHC 22C3 assay kit, whereas, atezolizumab requires the detection of PD-L1 expression on tumor cells by using the PD-L1 IHC 28–8 assay kit. The interpretation of results and scoring system are also different for each antibody and assay. For example, the reporting for the assay with the 28–8 antibody uses a 3 tiered scoring system for positive staining (1%, 5%, and 10%), while the assay with the 22C3 antibody uses a different 3 tiered scoring system (<1%, 1%–49%, and ≥50%)[17]. Therefore, it is necessary to develop a novel and universal biomarker to assess PD-L1 expression, rather than using individual markers and/or individual scoring system s.

The genetic characteristics of tumors provide the foundation for the development of personalized medicine in lung cancer patients. The current advances in targeted therapy and immunotherapy require accurate morphological subclassification of tumors. However, lung cancer, particularly NSCLC, consists of a heterogeneous group of tumors. This morphological heterogeneity may present diagnostic challenges, particularly when using small biopsy specimens. Clinically, most NSCLCs can be subclassified by evaluating histomorphological features using H&E (hematoxylin and eosin) stained sections. However, IHC markers are necessary for the accurate subclassification of lung cancers in daily practice. In this review, we will discuss the updated WHO (World Health Organization) guidelines for the classification of lung cancer, the utility and limitations of current IHC markers, and the prospective future of the discovery of potential IHC markers in lung cancer.

Current WHO guidelines for the subclassification of lung cancer

The WHO Classification of Lung Cancer provides diagnostic terminology and classification criteria for lung tumors [2, 3]. It also serves as a foundation for appropriate clinical management and the development of clinical trials. Based on molecular advances and the clinical demand for the accurate subclassification of lung cancer, the WHO and the IASLC (International Association for the Study of Lung Cancer) have updated their guidelines in 2015 [2–3]. These new guidelines have had significant impact on daily practice and patient care.

Major updates in the current guidelines include: (1) the molecular and immunohistochemical characterization of tumors and the accurate subclassification of lung cancer for personalized medicine, (2) the reclassification of “bronchioloalveolar carcinoma” into several different subtypes, including adenocarcinoma in situ, minimally invasive ADC, and ADC with lepidic growth pattern, (3) the subclassification of SqCC into basaloid, keratinizing, and non-keratinizing types, and the requirement of immunohistochemical evidence of squamous differentiation in non-keratinizing SqCC, (4) the change in diagnostic terminology for several tumors, including “sclerosing hemangioma” to “sclerosing pneumocytoma”, and “hamartoma” to “pulmonary hamartoma”, (5) the addition of diagnostic entities based on molecular studies, including “*NUT* carcinoma”, “pulmonary myxoid sarcoma with an *EWSR1–CREB1* translocation”, “myoepithelioma and myoepithelial carcinomas with *EWSR1* gene rearrangements”, “epithelioid hemangioendotheliomas with *WWTR1–CAMTA1* fusions”, and “PEComatous tumors” to include “benign and malignant PEComa” and “lymphangioleiomyomatosis”.

Clinically, the majority of lung cancer patients present with locally advanced disease or with distant metastasis at the time of diagnosis, and surgical resection of the tumor for morphological diagnosis may not be an option. Several minimally invasive approaches can be used to obtain tumor tissue, such as endobronchial ultrasound-guided transbronchial fine needle aspiration (EBUS-TBNA), and computerized tomography (CT) or ultrasound guided transthoracic fine needle aspiration (Figure 1) [18, 19]. However, these minimally invasive approaches often yield only small biopsy specimens, which can make accurate diagnosis challenging and often requires additional ancillary studies [2–3]. Recognizing the difficulty of evaluating small biopsy specimens in comparison to resected tumor tissue, the WHO guidelines have also recommended the following: (1) a separate set of criteria for the subclassification of small biopsy specimens from those for resected tumor tissue, (2) the requirement for IHC markers for the subclassification of lung cancer, and (3) the use of the diagnostic term NSCLC in certain biopsy cases. Certain tumors can only be classified using resected tumor tissue, rather than using small biopsy specimens, such as “large cell carcinoma”, which lacks any distinct morphologic or immunohistochemical differentiation, and can only be assessed when the tumor is resected. Another example is lung ADC with solid growth pattern, which can be confused with non-keratinizing SqCC on small biopsy specimens. In certain small biopsy specimens, the subclassification of the tumor, in addition to the morphologic evaluation and immunohistochemical characteristics of the tumor, may be still difficult. For these reasons, the guidelines emphasize the critical role of IHC markers in the accurate subclassification of lung tumors in addition to morphological evaluation, particularly in small biopsy specimens (Figure 2).

The Utility and Limitations of current IHC markers in the accurate subclassification of NSCLC

Although the majority of NSCLC cases can be subclassified based on histomorphological examination using hematoxylin and eosin (H&E) stained slides, sometimes the accurate subclassification of a tumor may be difficult due to a variety of reasons. For example, resected tumor tissue may have extensive necrosis and lack viable tumor cells, or the biopsy specimen may have scant tumor cells and lack the characteristic architecture of the tumor. Furthermore, artifacts during specimen preparation may also pose challenges. The degree of tumor cell differentiation is another factor which may potentially affect accurate diagnosis [20, 21]. For instance, poorly differentiated carcinomas lack the specific phenotypes and morphological features of either glandular or squamous differentiation (Figure 2). In these cases, IHC study of the tumor is necessary for diagnosis and determination of tumor subtype.

In daily clinical practice, the most commonly used IHC markers for the classification of NSLCLs are TTF-1 (thyroid transcription factor-1), Napsin A, CK5/6, P63 and P40 [22–24]. These markers have different sensitivities and specificities. Our studies and those of others have demonstrated that these markers have overall sensitivities and specificities ranging from 70% to 100% for the identification of ADCs, and from 60% to 100% for the identification of SqCCs [22, 23]. However, there are still limitations and drawbacks when using these markers.

TTF-1 and Napsin A are expressed by pneumocytes and are most useful for the identification of glandular differentiation in ADC [20–24]. For many years, TTF-1 has been the predominant marker employed for the identification of a neoplasm of lung origin, demonstrating a sensitivity ranging from 75% to 80% for lung ADC [20–23]. TTF-1 is a member of the Nkx2 family of transcription factors, and is also expressed by other tumors and normal tissues, such as thyroid tissue and thyroid tumors, as well as neuroendocrine tumors (SCLC and carcinoid tumors) [20–23]. The expression of TTF-1 is also inversely correlated with the degree of tumor differentiation, i.e. poorly differentiated ADCs are less likely to express TTF-1 compared to well differentiated tumors [22]. Additionally, Napsin A has also been identified as a marker for the detection of lung ADC [24]. Napsin A is an aspartic proteinase involved in the maturation of the surfactant protein B in lung tissue [24]. It is abundantly expressed in the cytoplasm of normal type II pneumocytes and Clara cells, in addition to lung ADCs, however, it is also expressed by kidney proximal and convoluted tubule cells, and renal cell carcinomas [20, 21, 24]. Additionally, the expression of Napsin A has been shown to be potentially regulated by TTF-1 [24]. Studies using resected tumor tissue have shown that Napsin A has better specificity than TTF-1 for the determination of lung origin among well to moderately differentiated lung ADCs [21]. However, its expression is decreased among poorly differentiated lung ADCs [20, 21].

Both P40 and P63 are products of the *P63* gene on chromosome 3q27–29, and are expressed by the basal or progenitor cell layer of bronchial epithelium[2–5]. The full-length protein TAp63 (containing the N-terminal transactivation domain) can be identified using the antibody 4A4 (P63), and the N-terminal-truncated protein isoform of TAp63 can be identified using the antibody P40 [26].Both P63 and P40 are used for identification of squamous differentiation. The sensitivity and specificity of P40 have been reported to be100% and 98–100%, respectively, in identifying SqCCs in surgically resected specimens [25, 26]. By using tumor tissue microarrays (TMAs), we have demonstrated that P40 and P63 have sensitivities and specificities of 80.95% and 90.0% (P40) and 93.5% and 80.0% (P63) in identifying lung SqCC, respectively[27].Our previous study was also consistent with these findings in that P40 had a higher specificity and lower sensitivity than that of P63[23, 27, 32] .

Cross-reaction with other tissue types and tumors is a limitation of current IHC markers [20–25]. For example, TTF-1 is immunoreactive with normal pulmonary alveolar macrophages, whereas, both P40 and P63 are immunoreactive with normal bronchial basal epithelial cells (Figure 3) [27]. This non-specific immunoreactivity of IHC markers with normal cells should not be confused with tumor cells. In addition, individual IHC markers require multiple sections of tumor tissue for the test to be performed. It is not uncommon for tumor tissue to be exhausted by IHC studies leaving little tissue for molecular analyses, particularly when using small biopsy specimens. Other limitations of IHC markers include: (1) the time-consuming nature of IHC testing, which can result in prolonged turnaround times, significantly affecting the patient's clinical management, (2) the influence on the interpretation of IHC results by non-specific staining of non-neoplastic cells, such as Napsin A positivity in pulmonary alveolar macrophages, and P40 positivity in normal bronchial basal cells, and (3) the different staining patterns of different clones of the same antibody.

To improve the efficiency and accuracy of subclassifying NSCLC, several recent studies have investigated the potential utility of combining several IHC markers into a single marker [28–31]. A dual marker combining TTF-1 and Napsin A demonstrated 74% sensitivity and 87% specificity [28], and 74% sensitivity and 88–96% specificity [29] in the identification of lung ADC using fine needle aspiration (FNA) material. A dual marker of P63 and CK5 demonstrated 100% sensitivity and 100% specificity in the identification of lung SqCC using FNA material [30]. Lung tumor tissue microarray (TMA) data using a dual marker of TTF-1 and P40 demonstrated 93% sensitivity and 92% specificity in diagnosing SqCC [31]. A marker combining desmoglein 3 and CK5 demonstrated 100% sensitivity and 100% specificity in diagnosing SqCC [31]. All of these studies demonstrate that dual or triple markers have similar or better sensitivities and specificities when compared to individual markers. Additionally, combined IHC markers also have the advantage of using minimal tumor tissue for immunohistochemical subclassification, thereby leaving more tissue available for molecular studies.

Recently, we tested a novel triple marker in our practice to subclassify NSCLC [32, 33] The triple marker consists of TTF-1, Napsin A and P40 (Figure 4), and has been developed on an automated staining instrument (BenchMark Ultra, Roche and Ventana Medical Systems, Tucson, AZ). In our study, the triple marker demonstrated a similar sensitivity and specificity when compared to individual IHC markers. The triple marker has a sensitivity of 86.0% and a specificity of 100% in identifying lung ADCs, and a sensitivity of 100% and a specificity of 97.1% in identifying lung SqCCs [32, 33]. Our approach has demonstrated that use of a combined IHC marker is a cost-effective method for subclassifying lung cancer in daily practice. Furthermore, the triple marker improves turnaround time and has the advantage of using minimal tumor tissue. It is now used routinely in our daily operation. The utility of the triple marker is summarized in Figure 5. However, the non-specific staining of normal pulmonary cells, such as alveolar macrophages and bronchial basal cells, remains a limitation of the triple marker. Therefore, the discovery of new markers is necessary to fulfill clinical demand for the accurate subclassification of NSCLC for the purposes of targeted therapy and personalized medicine.

Prospective future directions

Lung cancer development and progression are multistep processes [34]. They are characterized by aberrant genetic changes and protein expression, which subsequently lead to phenotypic transformation of cells and progression of the tumor [34–36]. This process involves complex intracellular signaling pathways and various cellular proteins [34]. In addition to the genetic characterization of lung cancer, it is also necessary to address the need for protein biomarkers for the accurate subclassification of lung cancer, as well as for monitoring disease progression and treatment response [34–36].

Over the past decade, numerous studies have been published related to discovery of candidate biomarkers in lung cancer. For example, a keyword search query in PubMed using “protein biomarkers in lung cancer” , yields more than twenty-thousand articles. Of this large number, more than thirteen-thousand articles are related to diagnostic biomarkers, and more than three-thousand articles are related to prognostic biomarkers. These studies

represent tremendous effort in the discovery of potential biomarkers for the detection, classification and progression monitoring of lung cancer. Using advanced molecular technologies and proteomics, numerous studies have reported candidate biomarkers for lung cancer. These state-of-the-art technologies provide new platforms not only for the systematic study of intracellular proteins, but also for the characterization of the complex microenvironment of lung tissue. However, none of these biomarkers have been widely used in daily practice. Recently, several markers have been studied for the identification of ADC and SqCC. For example, it has been reported that SPATS2 has a sensitivity and specificity of 67% and 100% for the identification of SqCC, and ST6GALNAC1 has a sensitivity and specificity of 67% and 100% for the identification of ADC when using resected tumor tissue [37]. The utility of these candidate biomarkers still needs to be validated in well-designed, large scale studies.

In biomarker discovery and potential clinical application, it is important to use carefully selected clinical specimens in both the discovery process and subsequent validation phase. The potential success of biomarker discovery and application largely depends on the quality and availability of patient specimens. This requires a large number of carefully selected patient cohorts to determine the potential utility of the biomarker. Clinical validation of potential biomarkers must be conducted in a way to maximally avoid false positive and/or false negative results. For each candidate biomarker, robust and reproducible assays need to be developed and used in the validation phase.

When considering PD-L1 immunotherapy, three separate drug-specific assays are required for the clinical application of checkpoint inhibitors (Table 2). These FDA approved tests are either called companion (for pembrolizumab) or complementary (for atezolizumab and nivolumab) tests [15–17]. These tests use 3 different antibodies and 3 sets of assay conditions . The companion test for pembrolizumab uses the Dako/Agilent 22c3 assay, whereas, the two complementary tests for atezolizumab and nivolumab use the Ventana SP142 assay and the Dako/Agilent 28–8 assay, respectively. This practice has potential drawbacks. For example, the oncologist has to be aware of which test to order for a specific inhibitor. If no clinical information is provided, pathology personnel must spend time determining which test to perform. Furthermore, different assays require different scoring systems [15–17] which may cause problems in the interpretation of results. Therefore, a single test using a novel marker is needed to unify the detection of PD-L1 expression in tumor cells in order to improve patient care.

In summary, targeted therapies and immunotherapy have progressed rapidly, however, the overall progression-free survival rate of lung cancer patients is still suboptimal. The current WHO guidelines emphasize the importance of the accurate subclassification of NSCLC for personalized medicine. In order to improve clinical outcomes for lung cancer patients, biomarkers for the accurate subclassification of lung cancer and for monitoring response to targeted therapy, are urgently needed. Recent advances in molecular techniques have significantly improved the identification and validation of potential biomarkers in experimental settings. Although select candidate biomarkers have been studied and evaluated using clinical specimens, further improvement of the workflow and validation process in large scale cohorts remains necessary. Finally, the lack of validation of prognostic

markers is also a concern in clinical practice, therefore, clinical specimens and patient cohorts should be selected carefully in both the discovery process and subsequent validation phase.

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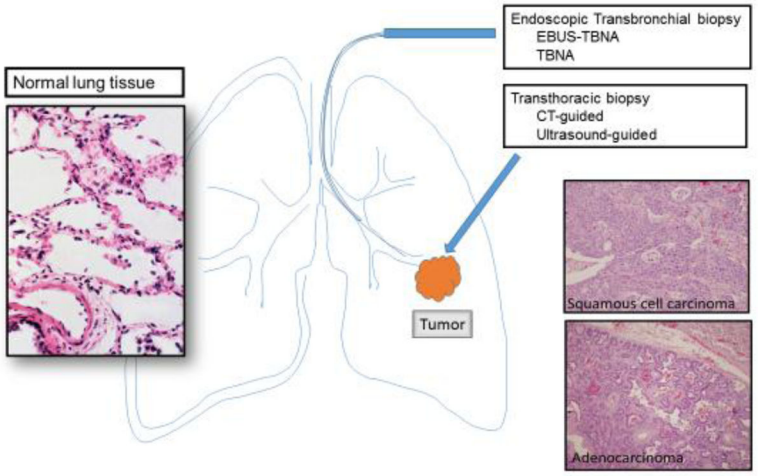

Figure 1.

Collection of Tumor tissue by FNA (fine needle aspiration) biopsies, and histomorphology of normal lung parenchyma, ADC and SqCC. Tumor tissue may be collected by endoscopic techniques, such as transbronchial FNA with or without ultrasound guidance, or transthoracic techniques with CT or ultrasound guidance.

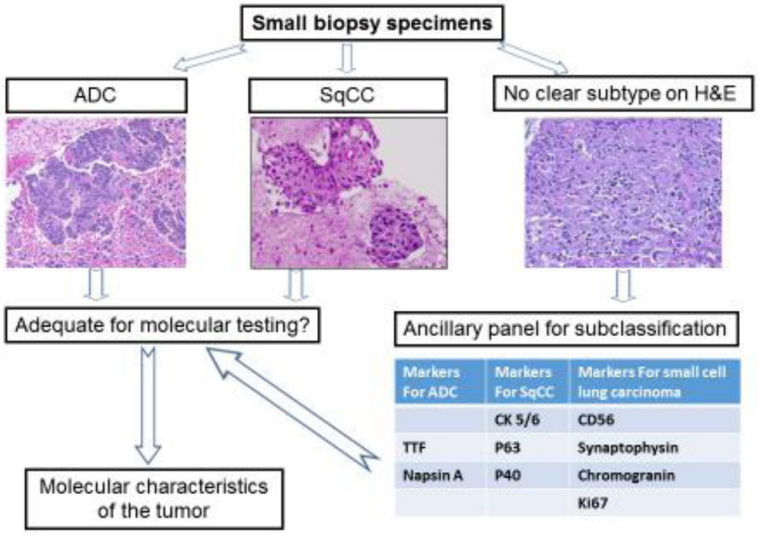

Figure 2.

Subclassification of small biopsy specimens. The diagnosis of ADC and SqCC can be made by finding evidence of glandular differentiation and cytokeration formation, respectively. However, the subclassification of a poorly differentiated carcinoma without obvious glandular or cytokeratin formation may require the use of a panel of IHC markers. The molecular characteristics of the tumor play a critical role for targeted therapy.

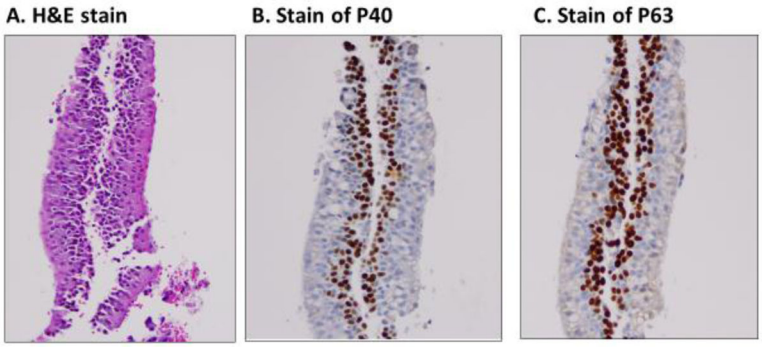

Figure 3.

Normal basal layer of bronchial cells is immunoreactive with P40 and P63. They should not be confused with squamous cell carcinoma. A, H&E stain of normal bronchial cells; B, immunostain of P40; and C, immunostain of P63.

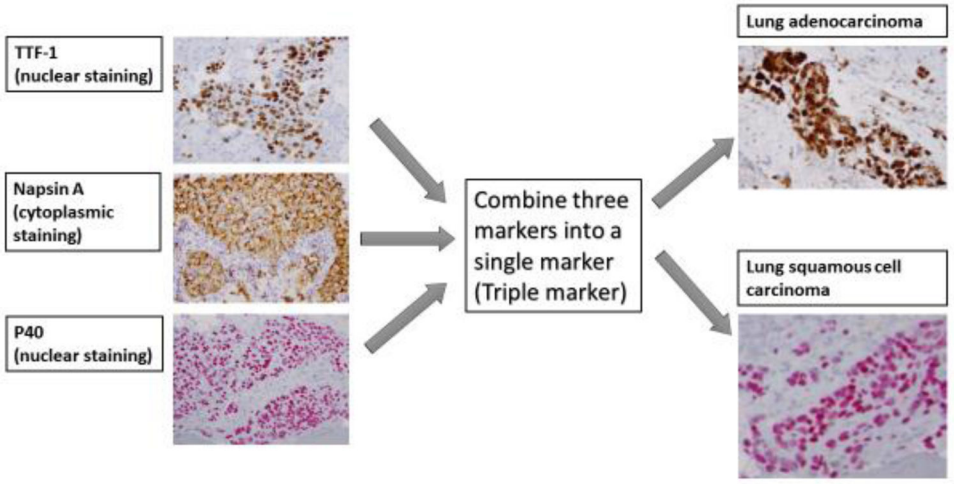

Figure 4.

The utility of a triple marker. We have combined three individual markers (TTF-1,Napsin A and P40) into a single marker to subclassify NSCLC.

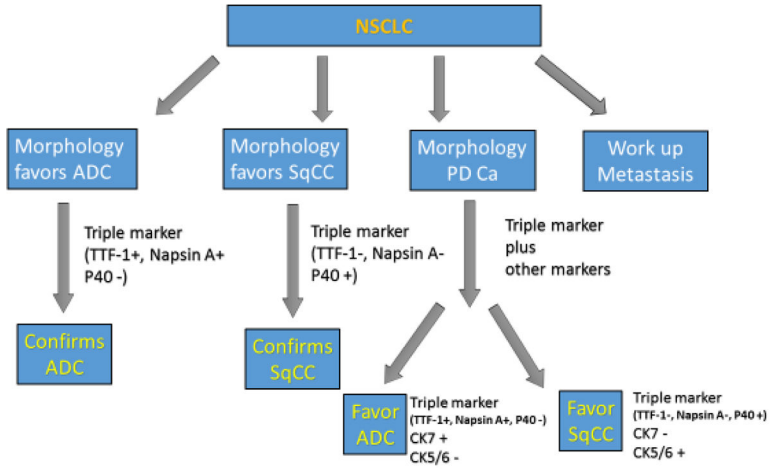

Figure 5.

The work flow for the differential diagnosis of NSCLC using the triple marker. Based on the histomorphological assessment of the specimen, different immunomarkers are performed.

Table 1

Major Somatic Alterations and Targeted therapies in Lung Cancer (adopted from reference #20)

Gene	PATHWAY	ABERRATIONS	%ADC	%SqCC	%SCLC	Drugs, approved and investigational
EGFR	RTK	Mutation Amplification	20–30% >20%	rare 7%		Erlotinib, Gefitinib, Afatinib (approved), Dacomitinib, Cetuximab, Necitumumab, Neratinib
ALK		Fusion with EML4 and other rare partners	3–13%			Crizotinib (approved), X-396, Ceritinib (LDK378); Ganetispib, AUY922, AT13387
MET		Mutation, amplification post-treatment with EGFR inhibitor	5% 20%			1. TKI: Tivantinib, Cabozantinib, Crizotinib 2. Monoclonal Ab: Onartuzumab, AMG102, Ficlatuzumab
ERBB2		Mutation, Amplification	2–4% 5–10%			Trastuzumab, Afatinib, Neratinib, MGHA22
ERBB3		Mutation		2%		MM-121
ROS		Mutation	1.5 %			Crizotinib, AT13387 (HSP90)
RET		Translocation with KIF5B and other genes	1–2%			Vandetanib, Cabozantinib ?
FGFR1		Amplification	1–3%	22%	6%	AZD4547, BGJ 398, BIBF 1120/nintedanib, dovitinib, HGS1036
DDR2		Mutation		3.8%		Dasatinib
IGF1R		Overexpression	ND	ND	95%	AXL1717, OSI-906
KRAS	RAS	Mutation	30%	5%		Selumetinib, Trametinib, MEK162, and BKM120, everolimus,sirolimus AUY922, BYL719,Reolysin MEK162
NF1			8–10%	11%		
HRAS				3%		
NRAS			<1%	<1%		
RASA1				4%		
BRAF	RAF	Mutations	6%	4%		Vemurafenib (only for V600E)
PIK3CA	PI3K	Mutation	rare	16%		BKM120, PX-866, GDC-0941
PTEN		Deletion	rare	8%		BKM120, PX-866, GDC-0941 (PI3K), MK-2206 (AKT)
AKT1,2,3			rare	16%(AKT3) 20% all		MK-2206
TSC1,2				6%		Everolimus, sirolimus, temsirolimus
LKB1	LKB1/AMPK	Mutation	15–30%	2%		Biguanide compounds
MDM2	TP53	Amplification	20%			Inhibitors of TP53 – MDM2 interaction
CDKNA2/p16INK4	RB1/ CDK	Deletions, silencing, mutation	>20%	70%		CDK inhibitors PD0332991, BAY1000394

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Gene	PATHWAY	ABERRATIONS	%ADC	%SqCC	%SCLC	Drugs, approved and investigational
MYC MYCN MYCL	Transcriptional regulators	Amplification	31%	rare	16%	Aurora kinase inhibitors, BH3 mimetics

Table 2

PD-L1 immunotherapies in lung cancers and PD-L1 assay conditions.

Checkpoint inhibitors	Target	Antibody Clone	Assay conditions	Detection System
Atezolizumab[1] [Tecentriq, Genentech/Roche, South San Francisco, CA]	PD-L1	SP142 [Ventana] Rabbit monoclonal	Ventana SP142 assay	Ventana Benchmark ULTRA platform
Durvalumab[2] [AstraZeneca, Wilmington, DE]	PD-L1	SP263 [Ventana] Rabbit monoclonal	Ventana SP263 assay	Ventana Benchmark ULTRA platform
Nivolumab[1] [Opdivo, Bristol- Muers Squibb, Lawrenceville, NJ]	PD-1	28–8 [Dako] Rabbit monoclonal	Dako/Agilent 28–8 assay	Dako Link 48 platform
Pembrolizumab[1] [Keytruda, Merck&Co Inc, Kenilworth, NJ]	PD-1	22C3 [Dako] Mouse monoclonal	Dako/Agilent 22c3 assay	Dako Link 48 platform
N/A	N/A	E1L3N[3] [Cell Signaling] Rabbit monoclonal	PD- 1(E1L3N)XP from Cell Signaling	Leica Bond platform

[1] FDA approved therapy

[2] in clinical trials

[3] in investigation